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THE INFLUENCE OF ANTHRAHYDROQUINONE
AND OTHER ADDITIVES ON THE CONDENSATION
REACTIONS OF VANILLYL ALCOHOL

DONALD R. DIMMEL, DONALINE SHEPARD
AND THOMAS A. BROWN

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Donald R. Dimmel, Donaline Shepard and Thomas A. Brown
The Institute of Paper Chemistry
Appleton, WI 54912

GENERAL SUMMARY

This paper, which has been submitted to The Journal of Wood Chemistry and Technology, describes work taken from Project 3475-2 (formally 3370). This project, entitled "Reactions of Pulping and Bleaching - Delignification Reactions," is concerned with how anthrahydroquinone (AHQ), the reduced form of anthraquinone (AQ), functions as a delignification catalyst. One way to promote delignification is to inhibit the condensation of lignin fragments. This paper describes how AHQ and other additives affect the condensation reactions of vanillyl alcohol, a lignin model, and of dioxane lignin, an isolated lignin of molecular weight 11,000.

Our results indicate that AHQ, but not AQ, can retard lignin-like condensation reactions. Exactly how it accomplishes this was the point of several experiments described in the paper; however, no firm conclusions could be drawn from the data since we still lack fundamental knowledge about the condensation process itself. Based on our work and previously published results from other laboratories, it appears that AHQ promotes delignification reactions by (1) promoting lignin fragmentation reactions and (2) retarding undesirable lignin condensation reactions.

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ABSTRACT

Vanillyl alcohol, a simple lignin model, has been heated with alkali under a variety of conditions and in the presence of several additives. The level of condensed products, principally dimers and trimers, has been determined in each case. Some additives, such as sulfide and anthraquinone, showed few differences from the control. Other additives, such as anthrahydroquinone, dithionite and 3,5-dinitrobenzoic acid, greatly depressed the levels of condensation products. The detection of a minor product, a head-to-head dimer, suggests some radical intermediates are present under these reaction conditions. The degree of condensation and ratio of products was quite temperature dependent. The influence of selected additives on the condensation reactions of a dioxane lignin has also been studied.

INTRODUCTION

The delignification of wood can be thought of as involving two primary reactions: first, a set of fragmentation reactions in which high molecular weight lignin is degraded into smaller units, some of which are water soluble and pass from the wood cellular structure to the cooking liquors, and second, condensation reactions in which lignin and/or lignin fragments combine to form high molecular weight material containing new types of bonds.^{1,2} This latter process is an undesirable one in that the condensed lignin is probably more resistant than the native lignin to solubilization and may contribute to "residual" lignin (that lignin which is the most difficult to remove during pulping).²

One of the principal benefits of employing catalytic amounts of anthraquinone (AQ) during alkaline pulping is rapid delignification rates.³ Anthrahydroquinone (AHQ), which is formed during pulping by the reaction of AQ with wood carbohydrates,^{4,6} has been shown by a number of research groups to promote fragmentation reactions of lignin model compounds.⁷⁻¹⁰ We would like to describe here our efforts to show that AHQ can also inhibit condensation reactions in a simple lignin model, namely vanillyl alcohol. The significance of this finding is that it predicts that pulping to low residual lignin contents should be possible in the presence of AQ; this has recently been realized in our laboratory.¹¹

RESULTS AND DISCUSSION

Vanillyl Alcohol Cooks

The condensation reactions of actual lignin would be expected to generate a very complicated product mixture. In an attempt to understand the chemistry of the process at a molecular level, we chose to study a model, vanillyl alcohol, which has some of the essential features of typical lignin. Vanillyl alcohol (VA) in hot alkali should reversibly form a quinonemethide.³ Many of the reactions of lignin, including condensation reactions, are postulated to proceed through quinonemethide intermediates.^{1,2} The anticipated alkaline reactions of VA are shown in Fig. 1.

If AQ or AHQ were to favorably interfere with these anticipated condensation reactions, one might observe (a) less polymer formation in the presence of the additives, relative to a control, (b) reduction products, such as creosol (6), and/or (c) oxidation products, such as vanillin (7) and vanillic acid (8).

Dilute solutions of vanillyl alcohol in 0.5N NaOH, containing no additives (the control), equal molar amounts of AQ, 3 molar equivalents of glucose or combinations thereof, were heated at

173° for 2 hours, under mild agitation, in sealed titanium reactors. The purpose of the glucose was to reduce AQ to AHQ. Since glucose is rapidly destroyed by base at elevated temperatures,¹² there should be no continuous generation of AHQ from AQ during the course of the cook. Consequently, equal molar amounts, rather than catalytic levels, of AQ were used.

Two different work-up procedures, as outlined in Fig. 2, were employed. Analysis of the organic components in either the freeze-dried or precipitated residues were qualitatively the same.

Analysis of the underivatized products by high pressure gel permeation chromatography (GPC) was not successful. Columns which were capable of differentiating small polymers, i.e., combinations of μ -Bondagel and μ -Porasil, did not function properly with the solvents necessary to dissolve the products. The SynCrompak column used in earlier studies¹³ showed very few differences between product samples; this column is not able to distinguish molecular weight differences below 5000. Consequently, GPC analysis did not allow a distinction to be made as to the degree of polymerization in the control runs vs. the runs containing additives.

Direct gas chromatographic (GC) analysis of precipitated and freeze-dried products, showed very few signals; creosol, the expected reduction product of quinonemethide 3, was not observed. Product samples which were extracted with hot tetrahydrofuran (THF), derivatized by methylation with dimethyl sulfate to increase the volatility of phenolic components and then analyzed by GC showed many more signals. Figure 3 shows the gas chromatograms of three cooked samples, containing the same amount of added internal standard (I.S.).

The cooked sample which contained AHQ (actually AQ and glucose) showed substantially lower amounts of dimers and trimers. (Proof of these structures will follow.) The chromatograms of the control sample and the one containing only AQ as an additive had nearly identical levels of dimers and trimers (Fig. 3, A and B).

Approximate yields of 4 and 5% were calculated for the main dimer and trimer, respectively, in the control and AQ cooks by assuming a GC response factor of 1.0 for these materials relative to the internal standard. When only glucose was used as an additive (GC curve is not shown in Fig. 3), the yield of main dimer and trimer was 3 and 1.3%, respectively. For the glucose/AQ additive mixture the yields were 1.5 and < 0.5% for the dimer and trimer.

Although the GC method is not capable of showing low volatility products, such as small polymers, one could infer from the yields of the dimers and trimer that much less polymerization was occurring in the AHQ cook relative to the control and AQ cooks. Apparently, AHQ, but not AQ, is capable of depressing condensation reactions of vanillyl alcohol.

The GC analyses of derivatized precipitated products were similar to those of the freeze-dried products, showing the same trends as just discussed. Glucose, alone, as an additive to the vanillyl alcohol cooks caused some decrease in levels of dimers and trimers, but not nearly to the extent of the glucose-AQ (i.e., AHQ) combination. One can speculate that glucose or its by-products might capture quinonemethides in irreversible reactions, thereby lowering the concentration of quinonemethide (QM) species and interfering with condensation reactions. Possibly AHQ can behave in a similar manner to glucose.

Analyses of the underivatized cooked samples by proton nuclear magnetic resonance ($^1\text{H-NMR}$) also showed that the control and AQ runs gave similar products, but the AHQ run produced additional aromatic signals in the 7.3-7.6 δ region and a sharp signal at 4.3 δ . Because of the method of workup, i.e., prolonged air exposure and filtration, the new signals in the aromatic region cannot be attributed to AHQ. Condensation reactions should produce $\text{Ar-CH}_2\text{-Ar}$ units, which will appear around 3.7-4.0 δ .¹⁴ This same region also contains ArOCH_3 signals. The aromatic signals for phenols and aromatic ethers occur in the 6.5-7.1 δ region of the spectrum. In comparing the spectra of the cooked samples, one can

see that the AHQ sample has less relative intensity in the $\text{OCH}_3/\text{ArCH}_2\text{Ar}$ region than the other samples. This is another indication that less condensation reactions have occurred in the presence of AHQ.

What is the cause of the signals in the 7.3-7.6 δ region which are so strong in the AHQ system and so weak in the others? This region is characterized by unsubstituted aromatics or aromatics which have no strong electron withdrawing or releasing substituents. Consequently, in our case this region would have to represent a vanillyl alcohol stripped of its aromatic oxygens or an AQ type molecule in which one or both carbonyl groups have been modified.

Product Characterization

The general method of characterization of the vanillyl alcohol condensation products was by gas chromatography - mass spectroscopy (GC/MS), although the major dimer and trimer were also collected by preparative GC and NMR spectra were recorded. The full details of the mass spectral interpretation of the fragmentation patterns of condensed products is reported in the next paper.¹⁵

The three most prominent components directly following AQ in the gas chromatogram (Fig. 3) showed mass spectral molecular ions of 288, 288 and 332, which would correspond to methylated dimers of vanillyl alcohol (molecular weight 154). The molecular ions were quite intense, which is indicative of highly aromatic structures.

Based on the fact that a dimer of structure 9 had been previously isolated from a vanillyl alcohol alkali reaction¹⁶ and its molecular weight after methylation would be 288, we assumed that one of the dimers corresponded to this structure. Compound 9 was synthesized¹⁷ and methylated; the resulting product was identical to the most abundant dimer in GC retention time, mass spectrum and NMR. The structure of the other mass 288 dimer is unknown at this time. The third dimer component probably corresponds to structure

10, since methylation of 10 would give a species of molecular weight 332.

The long retention time component, referred to in Fig. 3 as a trimer, was assumed to be the methylated derivative of structure 11, based on a mass spectrum displaying an intense molecular ion at 438 and fragment ions at m/e 287 and 151 and on a NMR spectrum that possessed the proper chemical shifts and ratio of aliphatic to aryl protons.

During the course of our investigation, both Yoon, *et al.*,¹⁸ and Hemmingson and Leary¹⁹ have reported on the self-condensation reactions of vanillyl alcohol. These workers isolated dimer - pentamers by exhaustive column chromatography. The structures of the condensed products, principally as their acetate derivatives, were characterized by spectral means and elemental analyses. Dimers 9 and 10 and trimer 11 have been characterized by both of these groups and their data agree well with our own.

Yoon and coworkers¹⁸ report higher yields than we observed; this may be related to (a) assumptions made by us with regard to GC response factors and (b) differences in reaction temperatures - we worked at much higher temperatures. Temperature does have a dramatic effect. Heating vanillyl alcohol at 60° in alkali changed the ratio of dimers; under these conditions, 10 was the most abundant isomer. Possible mechanisms for the formation of dimer 9 and trimer 11 are shown in Fig. 4.

Other components which we have detected and characterized as part of the vanillyl alcohol condensation products are: methylated vanillyl alcohol, vanillin, dimer 12, QM-AHQ adduct 13¹³ and AQ. Authentic samples of each of these were either purchased or synthesized and were shown to have identical GC retention times and mass spectra to those found in the condensation product mixture. With reference to Fig. 3C, the adduct corresponds to the small signal between the dimer/trimer region, dimer 12 is a very small signal in the dimer region (better seen in subsequent

chromatograms) and VA and vanillin are part of the low retention time components.

The adduct 13 was peculiar to the AHQ cook; the others mentioned above were present in all the cooks. Except for the adduct, there were no products which gave clues as how AHQ was retarding condensation reactions. Vanillin, for example, was no more abundant in the additive runs than in the control. Reduction product creosol and oxidation product vanillic acid were not observed in the AHQ runs, even though extraction procedures were employed to specifically look for them.

The formation of dimer 12 suggests there is some radical character to the condensation process, since ionic intermediates would not be expected to couple in a head-to-head fashion. Several unusual dimer and trimer products have been observed by GC-MS using chromatography conditions different from that shown in Fig. 3. For example, the GC-MS data suggest¹⁵ structures which are isomers of 10 and 11 that possess biaryl linkages meta, rather than ortho, to the phenolic hydroxyl groups. It is difficult to imagine a simple carbanion process (as shown in Fig. 4) that will explain these products. Acidic self-condensation reactions of vanillyl alcohol have been reported to give these unusual products;¹⁹ perhaps they can form to some extent in our case during the mildly acidic workup of the VA cook samples.

Another compound which was expected to be a component of the VA condensation product mixture was 2-vanillylanthraquinone (14). This compound has been isolated in low yield from pulping liquors.²⁰ The VA/AHQ cook sample produced a weak spot on a thin layer chromatography plate displaying the same R_f value and fluorescent quenching characteristic as 14. The compound was not specifically observed by GC/MS.

Variations in the Cooking Procedures

In the vanillyl alcohol reactions described so far, we used equal molar amounts of AQ (or AHQ) and vanillyl alcohol. What

would happen if low levels of AHQ were used? To answer this question we repeated the vanillyl alcohol cooks at various levels of AQ in an inert atmosphere.

The data are given in Table 1. The areas of the GC signals corresponding to the major dimer and trimer products and to the adduct 13 were compared to the area of an internal standard signal (equated to 1.0). Most of the decrease in dimer and trimer levels occurred with just a 2.5% level of AHQ; larger amounts of AHQ, however, led to decreased levels of condensation products and increased levels of adduct. Another trend was also quite apparent, namely, the level of trimer fell off more rapidly than the dimer. Presumably, this is a consequence of having consecutive reactions.

The dramatic effect of low levels of AQ on the VA condensation reaction suggests a (redox) catalytic action. The question is "what species is present to complete the redox cycle"? Glucose is known to be rapidly consumed by warm alkali.¹² Possibly, the VA-AHQ reactions are very rapid and are, thus, able to benefit from unreacted glucose. Maybe glucose by-products play a role.

We have qualitatively noticed that AHQ is much more effective at quenching condensation reactions of VA at high temperatures than at low temperatures. The distribution of some of the condensation products also varies with temperature. The data in Fig. 5 present the analyses for vanillyl alcohol cooks done at various temperatures, with and without 1.0 equivalent of AHQ present. Clearly, temperature does have a strong influence on the extent to which AHQ depresses VA condensation reactions.

Figure 6 compares the gas chromatograms of the product mixtures obtained from control and AHQ runs done at 85°; surprisingly, the level of dimers and trimers in the AHQ run was equal to, or greater than, the control run. Clearly, under conditions when adduct formation was high, condensation product levels were also high. At 85°, quinonemethides, such as 3, should be present

in solution but their concentration should be reduced by the reversible²¹ reaction which they undergo with AHQ to generate adducts, such as 13. Lowering the relative concentrations of QMs appears to have had little effect on the degree of condensation. Alternative explanations seem in order.

TABLE 1

Vanillyl Alcohol Cooks^a

% AHQ ^{bc}	Relative Levels of Products		
	Dimer	Trimer	Adduct
0.0	3.5	8.5	--
2.5	1.4	1.3	0.2
5.0	1.3	0.9	0.5
10.0	1.2	0.7	0.2
20.0	1.1	0.4	0.7
40.0	1.1	0.2	0.8
100.0	1.0	0.2	0.7

^aTwo hours at 173°C, 30 mL of 0.5N NaOH, 154.0 ± 0.6 mg of vanillyl alcohol, titanium bombs, flushed with N₂ before sealing.

^bGenerated by adding the appropriate weight of AQ to the reaction mixture containing 540 mg of glucose. The percent is figured on a molarity basis (2.5% molarity basis - 3.3% on a weight basis).

^cComparing this percent to the percent used in pulping is difficult since (1) vanillyl alcohol has a lower molecular weight than the typical lignin monomer (138 to 172), (2) wood is only 25% lignin, (3) not all the lignin units in wood are capable of forming QMs, (4) most of the lignin units in wood that form QMs also further react by β-aryl ether cleavage and (5) vanillyl alcohol can only undergo condensation reactions.

Several other VA condensation reactions were run in which an additive other than AQ or AHQ was added. For example, VA was heated with alkali and sodium sulfide. Analysis of the resulting product by GC showed, in comparison to a control cook, somewhat reduced levels of dimers but higher levels of trimer. Under the

same conditions, AHQ showed a large reduction in dimer and trimer levels. Thus, it appears that sodium sulfide is not very effective at retarding VA condensation reactions. [The pH was 13.0-13.7 during the reactions; therefore, the sulfur may be present as a mixture of S^{-2} and SH^{-} .]

In contrast, sodium dithionite was an excellent additive for retarding VA condensation reactions. This fact prevented us from employing dithionite in the VA/AQ cooks for the purpose of generating AHQ in situ. Another compound which depressed VA condensation reactions is 3,5-dinitrobenzoic acid (DNBA). The data in Table 1 show the analysis of some vanillyl alcohol cooks done at 100° with and without 0.1 equivalent of DNBA and/or AHQ. (The 100° data of Fig. 6 and Table 2 for the AHQ runs differ significantly; this is probably related to the presence, in the one case, of glucose, a mild condensation retarder.)

What properties do AHQ, dithionite and DNBA have in common that makes them good inhibitors of vanillyl alcohol condensation reactions? Dithionite is a good electron donor; it is used in alkaline solution to convert AQ to AHQ^{-2} . The addition of 3,5-dinitrobenzoic acid to an alkaline solution of anthrahydroquinone dianion leads to a rapid discharge of the red color associated with AHQ^{-2} and the formation of AQ. Therefore, AHQ^{-2} is an electron donor relative to DNBA. Perhaps all three of these additives function as electron donors to, let's say, a quinonemethide, converting the latter to a form that does not readily undergo condensation reactions (see Scheme I). Significant quantities of vanillin were observed in the DNBA cook product mixture; perhaps DNBA is functioning strictly as an oxidation catalyst.

Condensation Reactions of Dioxane Lignin

Loblolly pine dioxane lignin of weight average molecular weight of approximately 11,000²² was heated with aqueous alkali, with and without additives present. The additives examined were AQ (10% by weight, relative to the dioxane lignin), glucose (100%

by weight) and a glucose-AQ combination. After heating (173°) for 45 minutes, the reaction mixtures were cooled, acidified and freeze-dried. The molecular weight profiles of the dioxane lignin and the various products were compared by gel permeation chromatography using a SynChropak GPC 100 column and dimethylsulfoxide (DMSO) as the solvent, Fig. 7. The molecular weight profile of the dioxane lignin by this method was in excellent agreement with the profile obtained by gel filtration through a Sephadex G-100 column.²²

TABLE 2

Gas Chromatographic Analysis of the Derivatized Product Mixture of Vanillyl Alcohol Cook Samples

Component	Relative Amount ^a for Various Cooks ^b			
	--	AHQ ^c	DNBA ^c	AHQ/DNBA ^c
Monomers	4.1	3.2	2.2	6.3
Benzil	1.0	1.0	1.0	1.0
AQ	--	0.9	--	small
Dimers	13.5	4.4	6.5	3.5
Adduct	--	2.3	--	--
Trimers	4.6	2.0 ^d	2.3 ^d	1.1 ^d
Tetramers	5.5	0.1	--	--

^aQuantities present relative to benzil internal standard assuming a 1:1 GC response factor.

^bCooks were done at 100°C, in glassware, under nitrogen, for 2 hours. At the conclusion, the samples were exposed to air, filtered to remove most of the AQ, and acidified to obtain the product. The product mixture was dissolved in THF and derivatized with Me₂SO₄/OH⁻ prior to GC analysis.

^cThe additive level was 0.1 equivalent relative to VA; AHQ was generated by the dithionite method and the excess dithionite removed.

^dThe retention time of this component differed from the control and, thus, may correspond to something other than a trimer.

All of the cooks produced lignin of higher molecular weight than the starting dioxane lignin; however, the cook containing AHQ (Fig. 7, curve E) appears to produce the least amount of higher

molecular weight condensation products. The effects of AHQ might have been more pronounced if we had designed the experiment to provide a continuous means of regenerating AHQ. The results shown in Fig. 7 qualitatively agree with the results of several other lignin/AHQ molecular weight studies.^{7,23-25}

Perhaps a note of caution should be added at this point concerning GPC data on lignin. Lignin has a large number of polar groups which can adhere strongly to column packings unless a very polar solvent is employed. Most GPC columns do not perform well when polar solvents are used. The SynChropak column used in this study has never been used to analyze lignin; its main application area has been in the analysis of proteins²⁶ and carbohydrates.²⁷ The column adsorbed some lignin, giving distorted shapes, when 20% aqueous dioxane was used as the solvent during the analysis of the cook products. The column appeared to function well with DMSO as the solvent.

Besides adsorption effects, GPC molecular weight profiles can be distorted by changes in chromophores when using an ultraviolet (UV) detection system. The assumption which is generally made is that the high molecular weight components absorb in the UV to about the same extent as the low molecular weight components. If however, an additive, like AQ, causes additional chromophores in one, but not all, molecular weight species, then the true molecular weight distribution will be distorted.

A third problem with GPC is that unwanted UV absorbing species can interfere with the analysis. Ideally all the AQ should be removed from the samples since it is a strong UV-absorbing low molecular weight material. In filtering to remove AQ, some insoluble lignin could be lost. When acidifying to collect a lignin precipitate, the low molecular weight, water soluble, lignin will be lost. There is evidence that AQ becomes bound to the alkali soluble lignin found in soda/AQ pulping liquors.²⁸ This should affect the UV absorbance of lignin in the low molecular weight range.

CONCLUSIONS

The condensation reactions of vanillyl alcohol are sensitive to reaction temperature and additives, such as AHQ, dithionite, 3,5-dinitrobenzoic acid and, to some extent, glucose. The reactions appear to be relatively insensitive to sulfide ion, AQ and QM-AHQ adduct levels.

The types of products observed did not provide much information about how condensation inhibitors perform. The major differences observed, except for yields of condensed dimers and trimers, were in the NMR spectra of the cooked samples. The alkaline reactions of dioxane lignin, followed by GPC molecular weight analysis of the products, suggests that AHQ is also capable of inhibiting condensation reactions in this system.

Before fully understanding the role of additives on condensation reactions, it may be necessary to reexamine the mechanism of these reactions. The ionic pathways presented in Fig. 4 may or may not best explain how vanillyl alcohol condensation products arise. To what extent are radical mechanisms important? Why do additives which are good electron donors or acceptors seem to inhibit this process?

It would appear that the rapid delignification rates which accompany pulping with AQ can be explained by a combination of AHQ acting to promote lignin fragmentation reactions and to retard lignin condensation reactions.

EXPERIMENTAL

Vanillyl Alcohol Cooks - General. Vanillyl alcohol cooks were done in titanium bombs with a capacity of approximately 50 mL. Titanium was chosen in order to avoid possible metallic ion interferences; in actual fact, the results of reactions performed in stainless steel reactors were qualitatively the same as the titanium ones. Bombs were charged with 154 mg (1 mmole) of vanillyl

alcohol and 30 mL of 0.5N NaOH and various amounts of additives, (AQ, glucose, sodium dithionite) as described below. Mixing and temperature control were provided by rotating the bombs in an oil bath at 173°C for 2 hours. At the end of the cook the bombs were cooled quickly under cold running water. The cooked samples were worked up by two different methods. In both methods the samples were first stirred in air to oxidize any AHQ^{-2} present to AQ.

Method A: The samples were acidified with concentrated HCl to pH 6-7, then freeze-dried.

Method B: Samples containing AQ were filtered to remove the AQ, acidified as above to pH 6-7, then extracted with ether. The ether solution was dried over anhydrous Na_2SO_4 , decanted from the drying agent, and the ether allowed to evaporate in air at room temperature.

A series of cooks was done at various temperatures. At each temperature, a control sample (154 mg VA and 540 mg glucose in 30 mL of 0.5N NaOH) and an AQ sample (154 mg VA, 540 mg glucose and 208 mg AQ in 30 mL of 0.5N NaOH) were cooked 2 hours in a titanium bomb. The four temperatures were 85°C, 115°C, 135°C, and 175°C. Samples were worked up by Method A above.

Vanillyl Alcohol Cooks with Additives. Standard VA cooks as described above were done in the presence of various additives. The additives included AQ (208 mg, 1 molar equivalent), or glucose (540 mg, 3 molar equivalents), or a combination of the two additives. One set of cooks was done with varying levels of AQ; the conditions and relative amounts are detailed in Table 1. In another set of cooks varying levels of sodium dithionite ($Na_2S_2O_4$, 90%, technical grade) were added to the standard VA cooks with and without AQ (208 mg, 1 molar equivalent) present. Dithionite levels were 47.9 mg of 90% pure material (0.25 molar equivalent), 95.9 mg (0.50 molar equivalent) and 191.8 mg (1.0 molar equivalent).

The 3,5-dinitrobenzoic acid (DNBA) additive runs were done in glassware at 100°, rather than titanium bombs at 173°. A control

and AHQ run were also done under similar conditions. A solution of AHQ^{-2} was prepared by stirring together AQ (0.41 g), $\text{Na}_2\text{S}_2\text{O}_4$ (0.37 g), and NaOH (0.31 g) in 100 mL water under nitrogen at 60°C for 45 minutes. The solution was cooled, acidified with concentrated HCl then filtered and washed with water under nitrogen to remove excess $\text{Na}_2\text{S}_2\text{O}_4$. Addition of 0.31 g NaOH and 100 mL water regenerated the AHQ^{-2} (characteristic deep red color). DNBA (0.41 g) was added to the prepared AHQ^{-2} solution. An immediate color change, red to grey to purple, was noted. Vanillyl alcohol (3.0 g) was added to the reaction mixture and stirred 3 hours at 100°C under nitrogen. The reaction mixture was cooled, acidified with concentrated HCl then extracted with ether. The ether solution was dried over anhydrous Na_2SO_4 and the solvent removed on a rotary evaporator. The analogous experiment without AHQ^{-2} was performed by stirring together DNBA (0.41 g) and VA (3.0 g) in 150 mL 1N NaOH for 3 hours at 100°C. The sample was worked up as in the AHQ^{-2} run.

Derivatization. Vanillyl alcohol cook samples were derivatized by methylation with dimethyl sulfate, $(\text{CH}_3)_2\text{SO}_4$. Freeze-dried samples (or ether extraction residues) were extracted with hot tetrahydrofuran (THF). Powdered sodium hydroxide (1.0 g) and 2 mL of dimethyl sulfate (dropwise addition) were added to the THF extract and the solution was stirred overnight at room temperature. Enough water was added to dissolve the solids and the solution heated at 60°C for 1 hour to destroy excess $(\text{CH}_3)_2\text{SO}_4$. The THF was evaporated simultaneously with a stream of nitrogen. The pH was adjusted to approximately 9 with sulfuric acid. The solution was extracted with chloroform, the chloroform dried over anhydrous Na_2SO_4 and concentrated on a rotary evaporator. The sample was transferred quantitatively to a 10 mL volumetric flask containing 1 mL of a standard benzil solution (2 mg benzil/mL CHCl_3) and diluted to volume with CHCl_3 .

Method of Analysis, Gel Permeation Chromatography (GPC).

Analyses by GPC were done using a Varian 8500 pump in conjunction with a Perkin-Elmer LC-55 UV detector.

Samples were first neutralized with HCl then dissolved in 25% dioxane/H₂O. Solutions were filtered and a 50 µL aliquot injected onto the columns. The mobile phase was 25% dioxane/H₂O with a flow of 25 mL/hour. The first set of columns tried was composed of a µ-Bondagel E500, a µ-Bondagel E125 and a µ-Porasil, 60Å, in series. Severe tailing of the peak on the low molecular weight end of the chromatogram was observed with these columns. Removing the µ-Porasil column from the series did not improve the tailing problem and resulted in loss of low molecular weight resolution.

A second set of columns tried included µ-Bondagel E1000, E500, E300, E125 columns in series. These columns did not perform well in our system and it was not possible to obtain reproducible results. An attempt to analyze samples which had not been neutralized in order to minimize loss of high molecular weight material, and hopefully improve reproducibility, failed due to the incompatibility of these columns with very polar materials.

Some samples were extracted with dimethylsulfoxide (DMSO) and 50 µL aliquots of these extracts analyzed using a SynChropak GPC 100 column with DMSO (15 mL/hour) as the mobile phase. This column was not able to distinguish molecular weights in the range below 5000 and therefore proved unsuitable for these analyses.

Method of Analysis, NMR. - Proton NMR spectra of various cook samples and synthesized reference compounds were run on a JEOL FX 100 spectrometer. The solvent was CDCl₃ with TMS as an internal standard.

Method of Analysis, GC and GC/MS. - Gas Chromatographic analyses of derivatized sample were done either on a Perkin-Elmer 3920 GC, using a 6' SE-30 (3%) on Chromosorb-W column and nitrogen

carrier gas flow of 30 mL/minute, with temperature programming of 120° to 250° at 8°/minute and detection by flame ionization (Fig. 3) or a Hewlett Packard 5840 GC, using a 2' OV 101 (2%) on Ultra-bond 20M column, with temperature programming of 150° to 225° at 10°/minute and detection by a Hewlett-Packard 5985 mass spectrometer (Fig. 6). The all glass GC/MS system employed helium (30 mL/minute) as the carrier gas, a jet separator at 250°, a source temperature of 200° and an ionization voltage of 70 eV when operated in the electron impact (EI) mode. Methane (30 mL/minute) was used as the carrier gas with a direct line to the source (200°), operating at 230 eV, when obtaining chemical ionization (CI) spectra.

Positive identification of several components of the VA cook samples was achieved by methylating known or synthesized compounds and comparing GC retention times and mass spectra. These compounds included vanillyl alcohol (1), vanillin (7), bis-(3-methoxy-4-hydroxyphenyl)methane (9), 1,2-di-(3'-methoxy-4'-hydroxyphenyl) ethane (12) and 10-methoxy-10-(3'-methoxy-4'-hydroxybenzyl)-9(10H)-anthracenone (13).²⁹ Two compounds were identified based on comparison to the data of Yoon¹⁸ and Hemingson,¹⁹ NMR spectra of collected samples and the mass spectral fragmentation patterns;¹⁵ these were 3-(3'-methoxy-4'-hydroxybenzyl)-4-hydroxy-5-methoxybenzyl alcohol (10) and 1,2-di-(3'-methoxy-4'-hydroxybenzyl)-6-methoxyphenol. Many of the lesser components of the product mixtures have been tentatively identified by means of EI and CI mass spectra.¹⁵

A few of the VA cook samples were analyzed by GC before derivatization and retention times compared to that of authentic creosol (6); no significant level of creosol was observed.

Method of Analysis, Preparative GC. The major dimer and the trimer from VA cooks were purified and collected by preparatory GC using an Aerograph 200 GC with a thermal conductivity detector. The collected fractions were analyzed by ¹H-NMR. The compound

corresponding to the VA dimer had several peaks in the 3.7 - 3.9 region $[\text{OCH}_3, \text{ArCH}_2\text{Ar}]^{14}$ and peaks in the 6.8 δ region (phenolic aryl). The relative areas of the aryl to alkyl signals were 3:8, methylated structure 9 predicts 3:7. For the collected fraction thought to be VA trimer the $^1\text{H-NMR}$ spectrum showed peaks at the same chemical shifts as for the dimer. The aryl:alkyl area ratio was 8:22, exactly what methylated structure 11 predicts.

Method of Analysis, Extraction for Vanillic Acid. - Several freeze-dried VA cook samples, with and without AQ, were extracted with saturated sodium bicarbonate solution. The bicarbonate soluble portion was acidified with concentrated HCl then extracted with ether. The ether extract was dried over anhydrous Na_2SO_4 and the solvent removed on a rotary evaporator. The residue was analyzed by IR and the spectrum compared to that of vanillic acid. No evidence for vanillic acid in cook samples was found based on the complete absence of absorptions at 750 cm^{-1} in the IR spectra of these residues.

1,2-(3',4'-Dimethoxyphenyl)ethane (12). - The synthesis of this compound depended upon the fact that dibenzyl compounds are a common by-product when benzyl Grignard reagents are made.³⁰ An ether solution of 10 g (53.6 mmoles) of 3,4-dimethoxybenzyl chloride³¹ was slowly added to a stirred suspension of 0.65 g (26.7 mg-atoms) of magnesium turnings in anhydrous ether. The reaction was very difficult to initiate. The reaction mixture was stirred at reflux temperature for 3.5 hours. Much of the magnesium remained unreacted. Carbon dioxide was bubbled through the solution and the mixture allowed to stand overnight at room temperature. The ether solution was decanted from the unreacted magnesium and washed successively with dilute HCl and water, dried over anhydrous sodium sulfate (Na_2SO_4) and the ether removed on a rotary evaporator. The residue (5.8 g) was an amber, viscous

liquid. Analysis of the residue by GC/MS indicated several components, one of which exhibited a strong 302 signal, corresponding to the molecular weight of coupling product 12. This component was approximately 18% of the mixture, and was the major product. Residual starting material comprised 46% of the mixture.

Bis-(3-methoxy-4-hydroxyphenyl)methane. - This compound (9) was prepared according to the method of Lindgren³² and had a mp of 99-102°C (lit. mp 101-104°C). The purified compound was derivatized by methylation with dimethyl sulfate and the derivative analyzed by GC/MS and NMR: ¹H-NMR (CHCl₃) δ 3.33 (s, 2, CH₂), 3.84 (s, 12, OCH₃) and 6.8 (m, 6, aryl); ¹³C-NMR (CHCl₃) PPM 41.0 (CH₂), 56.0 and 56.2 (OCH₃), 111.4 (C-2), 112.4 (C-5), 120.8 (C-6), 134.4 (C-1), 148.0 (C-4) and 149.6 (C-3); MS (70 eV) m/e (%) EI 288(100), 257(6) and 151(11) and CI 317(12), M + 29, 289(79), M + 1, 288(10), M, 287(5), M - 1, 151(100).

ACKNOWLEDGMENTS

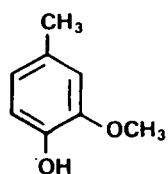
We would like to thank E. W. Malcolm and members of our Research Advisory Committee for their suggestions and support. We also appreciate the assistance of B. I. Fleming in determining the 2-vanillylanthraquinone in our cook samples.

REFERENCES

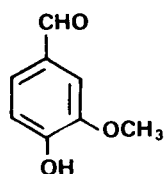
1. J. Martin, in Lignins, Chap. 16, K. V. Sarkanen and C. H. Ludwig (ed.), Wiley Interscience, New York, 1971.
2. J. R. G. Bryce, in Pulp and Paper, Chemistry and Chemical Technology, J. P. Casey (ed.), Wiley Interscience, New York, 1980, p. 428-429. Also W. G. Glasser, ibid, p. 69-76.
3. H. H. Holton, Pulp Paper Can., 78, T218 (1977) and many subsequent reports of recent years.
4. K. Iiyama, A. G. Kulkarni, Y. Nomura and J. Nakano, J. Jap. Wood Res. Soc., 24, 766 (1978).

5. G. L. Simard, J. M. Genco, and K. E. Henriksson, Jr., *Tappi* 62 (5), 91 (1979).
6. B. I. Fleming, G. J. Kubes, J. M. MacLeod and H. I. Bolker, *Tappi* 62 (7), 55 (1979).
7. J. R. Obst, L. L. Landucci and N. Sanyer, *Tappi* 62 (1), 55 (1979).
8. T. Yaguchi, S. Hosoya, J. Nakano, A. Satoh, Y. Nomura and M. Nakumura, *J. Jap. Wood. Res. Soc.*, 25, 239 (1979); *Japan Tappi*, 33, 666 (1979).
9. H. Aminoff, G. Brunow, G. E. Miksche and K. Poppius, *Paperi Puu*, 61, 441 (1979).
10. J. Gierer, O. Lindeberg and I. Noren, *Holzforschung*, 33, 213 (1979).
11. T. J. McDonough and V. J. Van Drunen, *Tappi*, 63 (11), 83 (1980).
12. J. W. Green, I. A. Pearl, K. W. Hardacker, B. D. Andrews and F. C. Haigh, *Tappi*, 60 (10), 120 (1977).
13. D. Dimmel, D. Shepard, T. Brown, and R. McKelvey, 1979 Canadian Wood Chemistry Symposium, Harrison Hot Springs, British Columbia, Canada, September 19-21, 1979.
14. Y. Nakai and F. Yamada, *Org. Magn. Reson.*, 11, 607 (1978).
15. D. R. Dimmel and D. Shepard, *J. Wood Chem. Technol.*, 1, ____ (1981).
16. J. M. Harkin, in *Lignin Structure and Reactions*, R. F. Gould (ed.), *Amer. Chem. Soc., Adv. Chem. Series*, 59, 65-74 (1966).
17. B. O. Lindgren, *Acta Chem. Scand.* 17, 2199 (1963).
18. B. H. Yoon, M. Okada, S. Yasuda and N. Terashima, *J. Jap. Wood Res. Soc.*, 25, 302 (1979).
19. J. A. Hemmingson and G. Leary, *Aust. J. Chem.*, 33, 917 (1980).
20. T. J. Fullerton and B. I. Fleming, *Svensk Papperstid.*, 83, 396 (1980).
21. D. R. Dimmel and D. Shepard, *Amer. Chem. Soc. National Meeting, Cellulose Division, Las Vegas, August 25, 1980.*
22. T. E. Crozier, D. C. Johnson and N. S. Thompson, *Tappi*, 62, 107 (1979).
23. B. I. Fleming, G. J. Kubes, J. M. MacLeod and H. I. Bolker, *Tappi*, 61 (6), 43 (1978).
24. A. Farrington, P. F. Nelson and N. Vanderhoek, *Appita*, 32, 300 (1979).

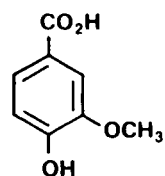
25. K. Iiyama and J. Nakano, ACS/CSJ Chemical Congress, Cellulose Division, Honolulu, Hawaii, April 1979.
26. T. D. Schlabach, A. J. Alpert and F. E. Regnier, Clinical Chem., 24, 1351 (1978).
27. H. G. Barth and F. E. Regnier, J. Chrom. Science, 192, 275 (1980).
28. W. H. Algar, A. Farrington, B. Jessup, P. F. Nelson and N. Vanderhoek, Appita, 33, 33 (1979).
29. Full details will be reported soon; preliminary details are given in Reference 13.
30. E. Berreby, J. P. Morizur and J. Wieman, Bull Soc. Chem. Fr., 3529 (1967).
31. J. F. Ginos, J. Org. Chem., 40, 1191 (1975).
32. B. O. Lindgren, Acta Chem. Scand., 17, 2204 (1963).



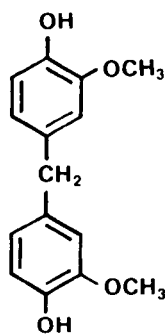
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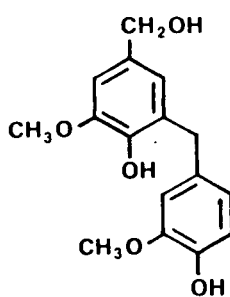
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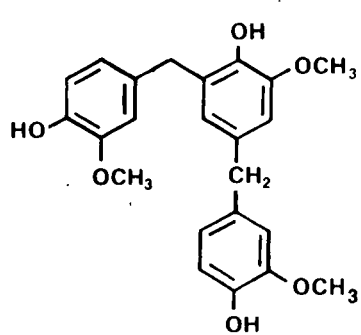
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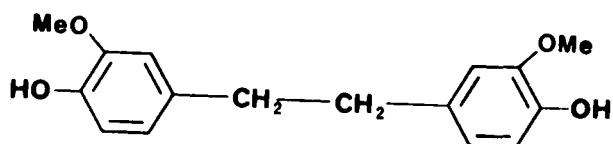
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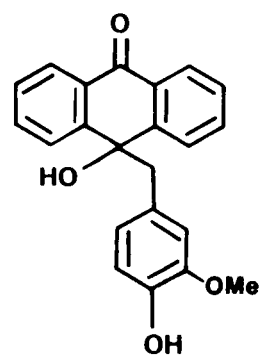
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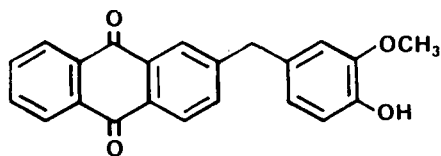
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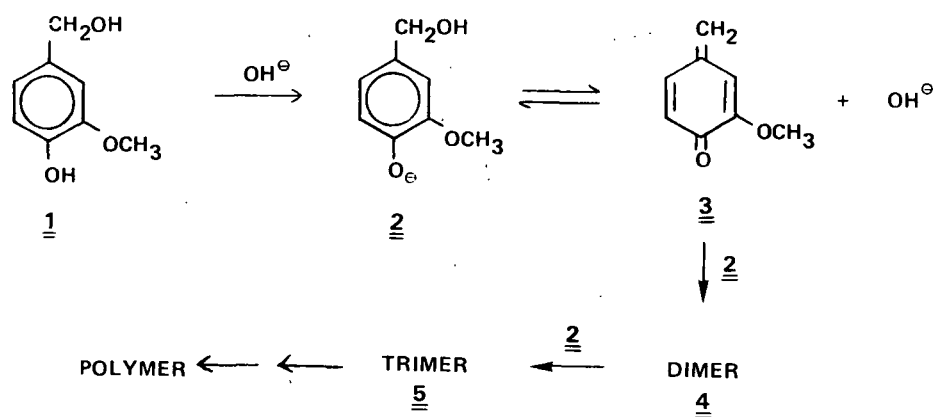


Figure 1. Possible Condensation Reactions of Vanillyl Alcohol

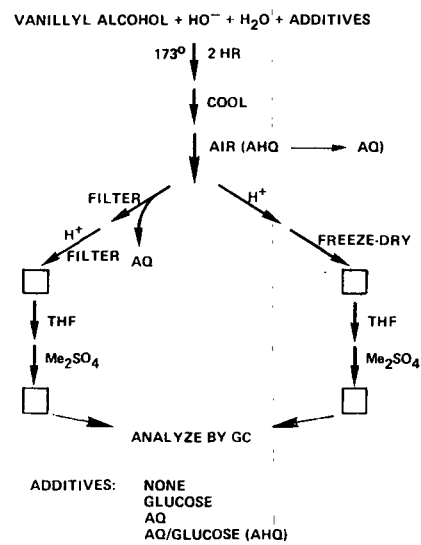


Figure 2. Outline of the Procedures Used in the Vanillyl Alcohol Reactions

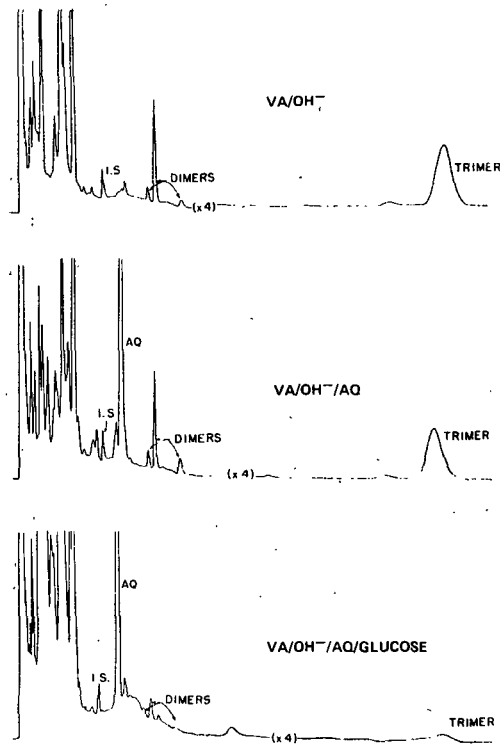


Figure 3. Reproductions of the Gas Chromatograms Obtained from Derivatized Products of Three Vanillyl Alcohol (VA) Cooks

PROBABLE MECHANISMS OF FORMATION AND STRUCTURES FOR THE
MAJOR VANILLYL ALCOHOL CONDENSATION PRODUCTS

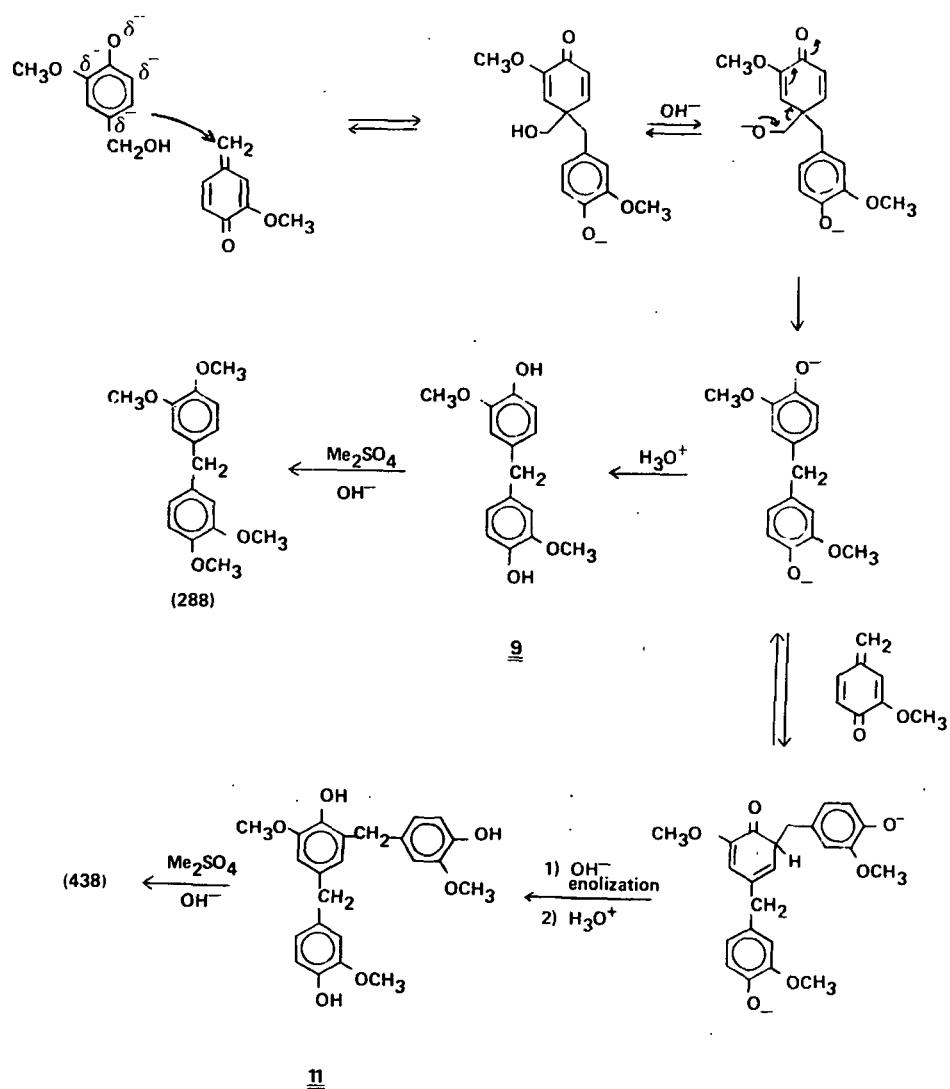


Figure 4. Possible Mechanisms of Formation for the Major Vanillyl Alcohol Condensation Products

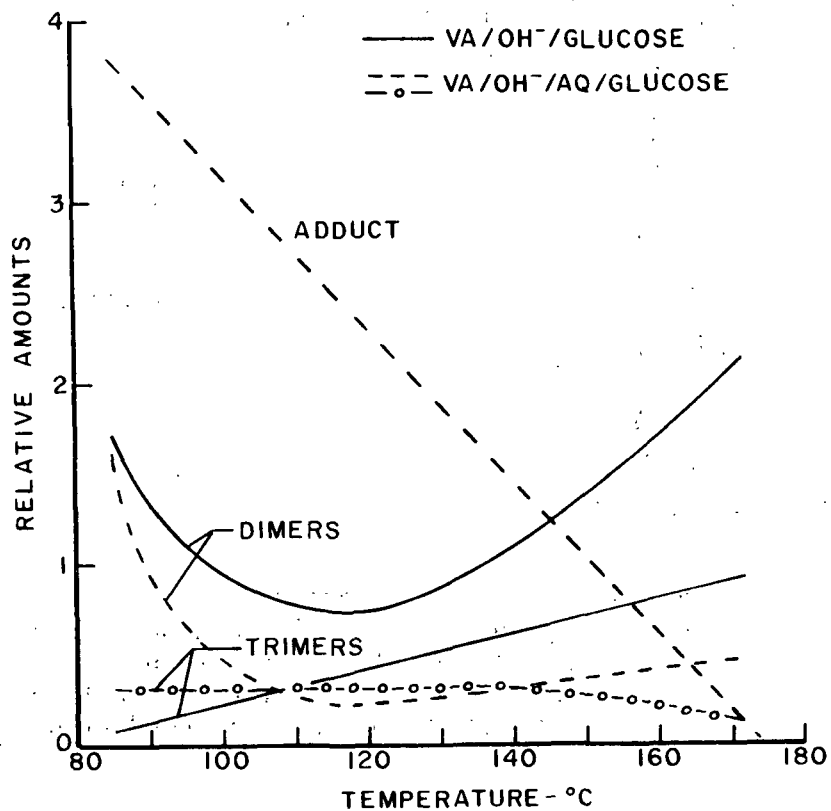


Figure 5. The Effect of Temperature on Product Concentrations, Relative to a Benzil Internal GC Standard, for Some Vanillyl Alcohol Cooks. The AHQ was Generated in situ by the Presence of AQ and Glucose at a 1.0 Equivalent Level (Relative to VA)

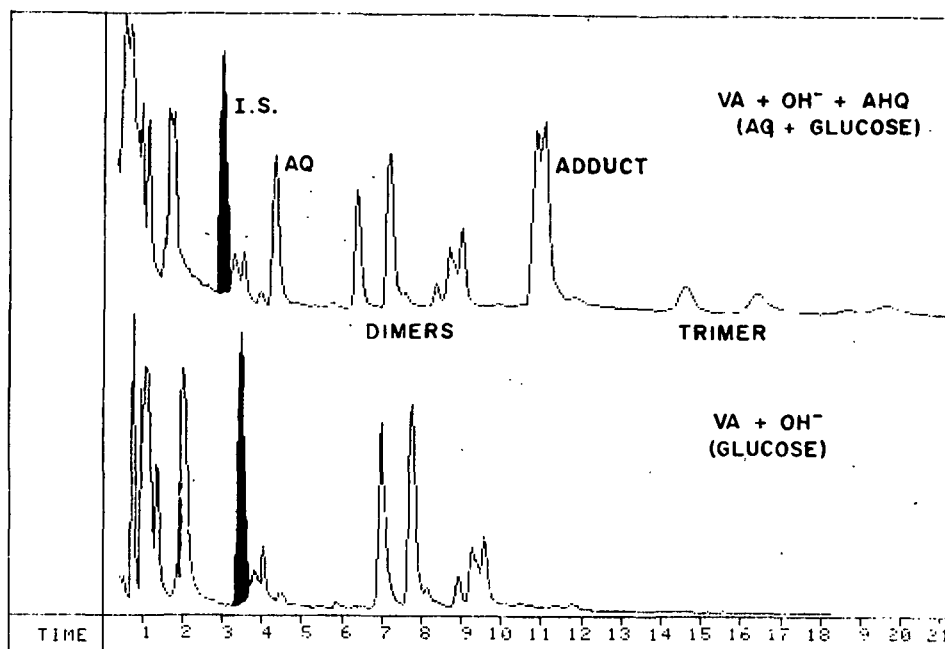


Figure 6. Comparison of the Gas Chromatograms of the Derivatized Products Obtained from Vanillyl Alcohol Cooks, with and without AHQ, at a Reaction Temperature of 85° for 2 Hours. The Retention Times Differ Slightly Due to Slightly Different Chromatographic Conditions

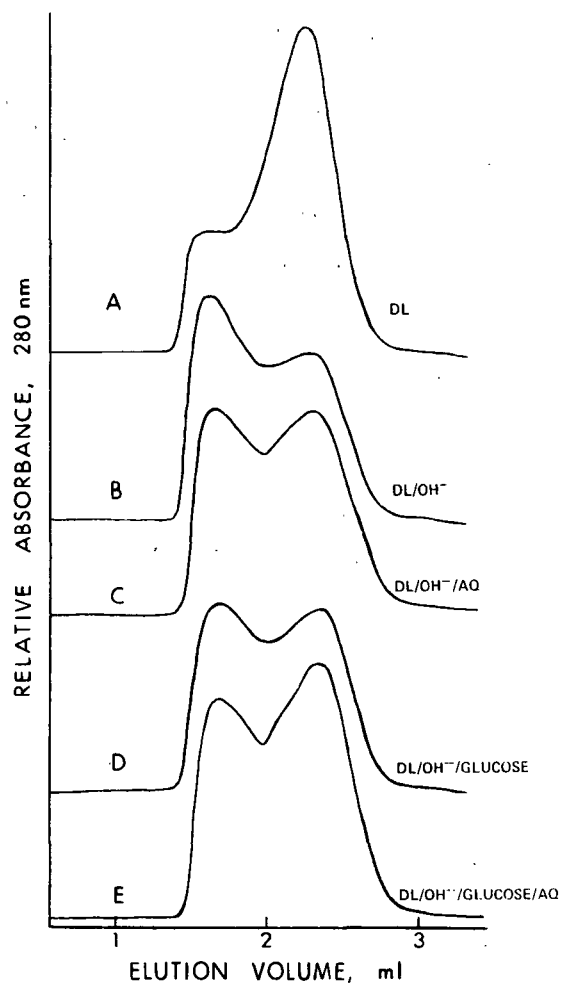
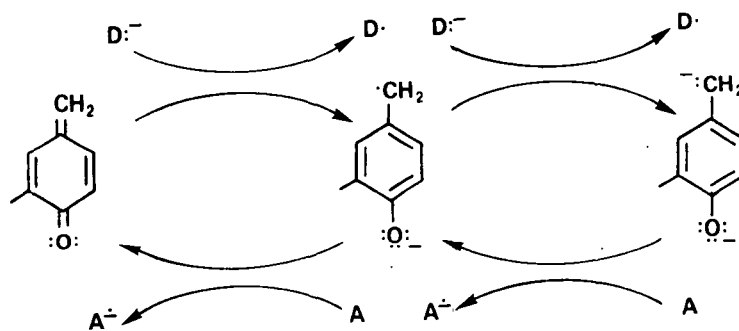


Figure 7. Gel Permeation Chromatograms of Dioxane Lignin (DL) and its Reaction Products with Alkali and Some Additives

SCHEME I



$\text{D} =$ electron donor
 $\text{A} =$ electron acceptor